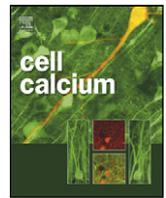




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Review

Activity-dependent calcium signaling and ERK-MAP kinases in neurons: A link to structural plasticity of the nucleus and gene transcription regulation

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ABSTRACT

Activity-dependent gene expression is important for the formation and maturation of neuronal networks, neuronal survival and for plastic modifications within mature networks. At the level of individual neurons, expression of new protein is required for dendritic branching, synapse formation and elimination. Experience-driven synaptic activity induces membrane depolarization, which in turn evokes intracellular calcium transients that are decoded according to their source and strength by intracellular calcium sensing proteins. In order to activate the gene transcription machinery of the cell, calcium signals have to be conveyed from the site of their generation in the cytoplasm to the cell nucleus. This can occur via a variety of mechanisms and with different kinetics depending on the source and amplitude of calcium influx. One mechanism involves the propagation of calcium itself, leading to nuclear calcium transients that subsequently activate transcription. The mitogen-activated protein kinase (MAPK) cascade represents a second central signaling module that transduces information from the site of calcium signal generation at the plasma membrane to the nucleus. Nuclear signaling of the MAPK cascades catalyzes the phosphorylation of transcription factors but also regulates gene transcription more globally at the level of chromatin remodeling as well as through its recently identified role in the modulation of nuclear shape. Here we discuss the possible mechanisms by which the MAPKs ERK1 and ERK2, activated by synaptically evoked calcium influx, can signal to the nucleus and regulate gene transcription. Moreover, we describe how MAPK-dependent structural plasticity of the nuclear envelope enhances nuclear calcium signaling and suggest possible implications for the regulation of gene transcription in the context of nuclear geometry.

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1. Introduction

Individual neurons are born within the first weeks of life and persist throughout the lifetime of the animal, which can for some species exceed 100 years. During this time the cell has to undergo many experience-driven adaptations, which are reflected by remodeling of the dendritic tree, axonal growth and formation and elimination of synapses [1–7]. Such processes are controlled by electrical activity and require transcription of appropriate sets of genes in the nucleus [7,8]. The major intracellular messenger that transduces electrical activity into gene expression is calcium [9,10]. During development, the maturation of excitatory synapses depends on calcium influx through synaptic *N*-methyl-D-aspartate receptors (NMDARs) which leads to local modifications such as insertion of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) into the

spine and rearrangement of the post synaptic density (PSD) [11,12]. Moreover, activity-dependent calcium-influx through NMDARs and voltage-gated calcium channels (VGCCs) is required to induce gene transcription events in the nucleus, which underlie widespread loss or pruning of dendritic spines and large-scale reorganization of the dendritic tree [13,14].

LTP and LTD are considered cellular correlates of learning and memory and involve rapid and persistent remodeling of synaptic connections. Rapid processes such as AMPAR insertion/removal, remodeling of the actin cytoskeleton and spine turnover are regulated by local calcium and its downstream kinases and are thought to be independent of gene transcription and nuclear calcium signaling [15]. Long-lasting adaptations however, require synthesis of new proteins that alter neuronal function and stabilize synapses at a longer time scale [7,8,16,17]. In order to decode the requirements of the synapse at the level of gene transcription, reliable delivery of the information about synaptic activity to the nucleus is essential. In principle, two strategies exist by which calcium can transform electrical activity into a nuclear gene transcription event: (1) calcium activates signaling molecules in the cytoplasm which carry

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the information to the nucleus via direct nuclear translocation or indirectly via signaling cascades, or (2) calcium itself crosses the nuclear envelope and acts directly within the cell nucleus.

In recent years many different calcium-dependent signaling pathways to the nucleus and their role in encoding the source and kinetics of intracellular calcium transients have been described [7,18–21]. Since calcium-dependent activation of gene transcription eventually requires the physical translocation of a signal from the cytoplasm across the nuclear envelope it is important to consider the accessibility of the nucleus for such signaling molecules. Small molecules such as calcium or calcium bound to calmodulin may enter the nucleus unhindered via diffusion through nuclear pore complexes [22–25]. However, larger molecules such as the calcium/calmodulin dependent kinases (CaMKs), protein kinase A (PKA) or the extracellular signal-regulated kinases 1 and 2 (ERK1/2) exceed the size limit for passive diffusion through the pore and thus require a mechanism that facilitates their nuclear translocation [26–29]. In the first part of this review we will consider the principal mechanisms by which calcium signals can be conveyed to the nucleus focusing on nuclear entry of calcium and on nuclear signaling of the ERK1/2-MAPK cascade.

In addition to direct activation of transcription factors recent evidence suggests that ERK1/2 can regulate chromatin remodeling – a higher-order mechanism of transcriptional regulation. ERK1/2-dependent phosphorylation of histone H3 and subsequent gene transcription have been described in many cell types and more recently these processes have been demonstrated to be important for synaptic plasticity and memory formation in different areas of the brain. Finally, recent evidence suggests that calcium and ERK1/2 act in concert at the level of structural remodeling of the nuclear envelope itself. Synaptic activity initiates deep infoldings of the nuclear membrane leading to a compartmentalized nucleoplasm, an increased surface area and amplified nuclear calcium signaling. In the second part of this review, we will summarize the current knowledge about neuronal ERK1/2 signaling and discuss the new nuclear functions of ERK1/2 in more detail. Moreover, we will provide suggestions how structural plasticity of the nucleus can impact on nuclear calcium signaling and activity-dependent gene transcription.

2. Nuclear signaling mediated by calcium

Several different transcription factors and their upstream kinases are regulated by calcium [18]. However, transcription is often not directly controlled by calcium ions but requires the involvement of specific signaling molecules and adaptors, which are activated by calcium inside or outside the nucleus. Thus, several routes exist by which calcium can relay a message to the nuclear transcription machinery. For example, the presence of nuclear calcium has been demonstrated as a requirement for the induction of activity-dependent, cAMP response element binding protein (CREB)-mediated gene transcription [10,30,31] and the relief from transcriptional repression via DREAM [32,33]. In addition, activation and nuclear translocation of other transcriptional regulators such as NF-AT4 and NF- κ B can be directly or indirectly induced by cytoplasmic calcium [34,35]. One of the most widely occupied routes is the calcium-mediated activation of protein kinase cascades in the cytoplasm such as CaMKs, PKA, MAPKs and their subsequent translocation into the nucleus [7,21,36,37]. The MAPK cascade has been especially well characterized in the context of calcium-dependent regulation of nuclear events. A large number of direct and indirect targets of this cascade have been found in the nucleus and are well described elsewhere [36,38–40].

The need for both nuclear calcium and indirect signaling to the nucleus via intermediate kinase cascades in order to induce

gene transcription was especially well documented for CREB, which is one of the best described transcription factors in neurons and is involved in learning and memory [41,42]. Interestingly, phosphorylation of Ser133 can be mediated by several different signal transduction cascades such as the mitogen- and stress-activated kinase 1 (MSK1) and ribosomal protein S6 kinase 2 (RSK2) but also by CaMKI, II and IV or PKA that convey information from cytosolic calcium to the nucleus [43]. Thus, phosphorylation of CREB on serine 133 through some of these pathways does not require nuclear calcium. However, additional calcium dependent phosphorylation of CREB and its co-activator CBP/p300 is necessary for the initiation of CREB-dependent gene transcription [10,30,31], which directly depends on nuclear calcium. This example aptly illustrates that both direct nuclear translocation of calcium itself and calcium-dependent nuclear signaling via kinase cascades such as the MAPK cascade are important for activity-dependent regulation of neuronal gene transcription. The accessibility of the nucleus for calcium and its downstream effectors is potentially therefore one determining factor for transcription efficacy.

3. Direct nuclear calcium signaling – pathways and mechanisms

How does calcium reach the nucleus? In the case of synaptic plasticity, activation of a few synapses is sufficient to induce persistent long-term potentiation through calcium-induced gene transcription. In a typical neuron these activated synapses can be widely distributed across the dendritic branches with distances of several hundreds of micrometers from the cell soma, which contains the nucleus. This raises the question how perinuclear calcium reaches sufficiently high concentrations to efficiently enter the nucleoplasm in order to bind and activate its nuclear targets if only a spatially confined population of synaptic NMDARs was activated in a dendrite distant from the soma. In principle, there are two ways by which synaptic activity can raise calcium concentrations in the cytoplasm: (1) influx through AMPARs, NMDARs, and VGCCs and (2) release from internal stores.

How can calcium signals arising from these sources be effectively transmitted to the nucleus? It is unlikely that synaptically generated calcium signals travel unassisted from their dendritic origin through the cytoplasm to the nucleus by simple passive diffusion. In fact, activation of NMDARs requires sufficient depolarization of the neuron, which is commonly achieved by back propagation of action potentials into the dendrite [44,45]. Moreover, NMDARs in their function as ion channels augment membrane depolarization and can in this way contribute to action potential generation [46]. Excitatory postsynaptic potentials (EPSPs) that travel down the dendrites towards the soma and back-propagation of action potentials into the soma and proximal dendrite will open L-type VGCCs which are preferably localized on the neuronal soma and proximal dendrites [47]. Opening of these channels generates robust somatic calcium transients, which have been shown to induce CREB phosphorylation, CREB-dependent upregulated gene expression, and transcription-dependent synaptic plasticity [48,49]. In addition, the activation kinetics of L-type VGCCs promotes different responsiveness to trains of EPSPs versus action potentials allowing a discrimination of different depolarizing stimuli [50,51]. The pattern of neuronal activity may therefore determine the shape of the VDCC-evoked somatic calcium signals. Their strategic localization and their activation kinetics thus support the idea that L-type VDCCs are the major source for synaptic activity-induced perinuclear calcium transients.

Such somatic calcium transients elicited near the nucleus may invade the nucleoplasm in an unconstrained way allowing precisely timed control of gene transcription events. Although it has been

widely debated in the field whether the nuclear envelope comprises a diffusion barrier for calcium ions (reviewed by [23,25]) it was recently demonstrated in hippocampal neurons that the rate of calcium flux into the nucleus is not limited by the nuclear envelope [24]. These findings clearly imply that timing and amplitude of somatic calcium transients will be faithfully relayed to the nucleoplasm for detection and decoding by calcium-sensing molecules. Notably, the effectiveness of calcium penetration into the nucleoplasm depends mainly on the surface-to-volume ratio, thus making calcium signaling more robust in nuclei exposing a relatively large surface to the cytosolic space. This is analogous to the importance of spatial considerations in the signaling of calcium microdomains around calcium channels in the plasma or endoplasmic reticular membranes [52,53]. We will discuss later in this review how structural plasticity of the nucleus may enhance this process in an activity-dependent manner.

The second strategy for the calcium-dependent conveyance of information between synapse and nucleus is inositol (1,4,5)-trisphosphate receptors (IP₃R)-mediated calcium release from internal stores. In this way 'calcium waves' can be generated that propagate from distal regions of their initiation towards the cell soma [54–56]. In neurons, IP₃R-dependent calcium release has been implicated in a wide variety of physiological processes including long-term synaptic plasticity, CREB-mediated gene transcription and neurite outgrowth [52,57–63]. In general, these calcium transients feature slower onset and decay kinetics and larger amplitudes than glutamate receptor (GluR) or L-type VGCC-evoked calcium signals making them ideally suited to bring about large, long-lasting calcium signals and robust gene transcription in the nucleus [54,55,64,65]. The endoplasmic reticulum, a major intracellular calcium store, has been proposed to form a continuum with the nuclear envelope in most cell types and therefore calcium can be released in very close proximity to the nucleus [26]. No consensus has been reached regarding the question whether calcium can be directly released into the nucleoplasm via IP₃Rs and ryanodine receptors (RyRs) or whether release occurs at the cytoplasmic face. It has been reasoned that in the latter case calcium could still very efficiently enter the nucleoplasm by fast diffusion through the nuclear pore complexes (NPCs) [22,66]. As a consequence, perinuclear calcium signals arising in the soma, which is largely occupied by the nucleus, have a high potential to reach the nucleoplasm rather than quickly dissipating throughout the cytoplasm and being extruded into the extracellular space or sequestered into organelles.

4. Indirect nuclear calcium signaling – the ERK1/2-MAPK cascade

Apart from direct delivery of calcium itself to the nucleus, cytoplasmic calcium transients generate nuclear signals indirectly via protein kinase cascades. Several of those complex calcium-activated signaling systems coexist in neurons. We will narrow our focus on the ERK1/2-MAPK cascade because of its central and universal importance in synaptic plasticity and memory formation in many species, brain areas and types of synapses. Moreover, recent discoveries that ascribe new functions to nuclear ERK1/2 signaling may reveal another layer of gene transcription regulation.

Aside from its activation by many signaling molecules such as brain-derived neurotrophic factor (BDNF), norepinephrine, dopamine, acetylcholine (ACh), histamine, estrogen, serotonin [67], the ERK1/2-MAPK cascade is a major target of synaptically evoked calcium signals. Alternative pathways have been suggested how NMDAR/VGCC-mediated calcium-transients activate Ras-GTP – the initial step in the cascade. Some studies have proposed that calcium/calmodulin complexes regulate cer-

tain Ras guanyl-nucleotide releasing factors (Ras-GRFs), which can activate Ras [68–70]. Others have implied a role for the calcium/calmodulin-dependent kinase kinase (CaMKK) and calcium/calmodulin-dependent kinase I (CaMKI) in the activation of ERK1/2 [21,71]. Synapse-specific activation of the ERK1/2-MAPK cascade downstream of NMDAR-mediated calcium signaling may be controlled by synGAP, a CaMKII-dependent Ras-GTPase activating protein, localized almost exclusively to the PSD [72]. In addition, there is also evidence suggesting that PKA and protein kinase C (PKC) signaling are required to yield physiologically relevant activity of ERK1/2 [37]. More recently, the scaffolding protein kinase suppressor of Ras (KSR1) was identified as a modulator of PKC-dependent ERK1/2-signaling, indicating that ERK1/2 activity may also be individually modulated by certain intracellular pathways [73].

ERK1 and ERK2 play a central role in neuronal signal integration being activated by receptors for multiple extracellular factors and neurotransmitters and control the activity of many different intracellular signaling substrates both in the nucleus and in the cytoplasm (Fig. 1). In neurons, the regulation of a number of these substrates occurs during the induction and maintenance of LTP and LTD where calcium is a key modulator of ERK1/2-activity. Consistent with a role of ERK1/2 in the remodeling of cytoskeletal elements, cytoplasmic activity of ERK1/2 was shown to modulate postsynaptic AMPA receptor trafficking and insertion [15,74–76] and to trigger the outgrowth and stabilization of postsynaptic spines [77,78]. Moreover, the activity-dependent translation of mRNAs, as well as their targeting to the site of LTP expression (e.g. a population of spines in a dendritic segment) has been shown to depend on ERK1/2 activity [78–80]. ERK1/2 can also phosphorylate the voltage-dependent K⁺-channel subunit Kv4.2 and thereby reduce its membrane-surface expression resulting in increased excitability of the soma and dendrites during LTP [81,82] (Fig. 1).

In addition to regulating cytoplasmic substrates involved in long-term synaptic plasticity, ERK1/2 signaling is also required for the activation of nuclear substrates during LTP induction. In this case, ERK1/2 propagates signals generated by synaptically evoked calcium into the cell nucleus, where it leads to the induction of gene transcription. The transcription factors, which are regulated by ERK1/2 control several different genes during synaptic plasticity. However, until now, the identity of only a few of these ERK1/2-dependent genes has become unveiled, including *zif268*, *bdnf*, *arc*, *c-fos* and *c-jun* [83–87]. This is because the promoter region of most genes contains several regulatory elements that are controlled by different kinases and thus, the magnitude of gene expression often depends on the combined activity of multiple signaling cascades as discussed above for CREB: nuclear ERK1/2-signaling is required but not sufficient to elicit robust, long-lasting CREB-dependent gene transcription [30,31]. ERK1/2-mediated nuclear signaling occurs either directly by phosphorylated ERK1/2 (pERK1/2) following its translocation from the cytoplasm to the nucleus or indirectly through downstream kinases that are phosphorylated by pERK1/2 in the cytoplasm before entering the nucleus (Fig. 1). In the case of CREB activation, phosphorylation of Ser133 on CREB is achieved downstream of either nuclear or cytoplasmic pERK1/2 [42]. Such downstream kinases include MSK1/2, which is exclusively localized in the nucleoplasm and therefore requires the presence of active MAPKs in the nucleus [88], and RSK2, which undergoes nuclear import following its phosphorylation by pERK1/2 in the cytoplasm [36] (Fig. 1). Other neuronal substrates of nuclear ERK1/2 signaling are the ternary complex factor Elk-1 [89,90] and the immediate-early genes (IEGs) c-Jun [91] and c-Fos [92]. These signaling pathways have been known for a long time and are well described elsewhere [38,93].

Recent work has revealed a role for nuclear ERK1/2 signaling in chromatin remodeling, a mechanism for regulating gene tran-

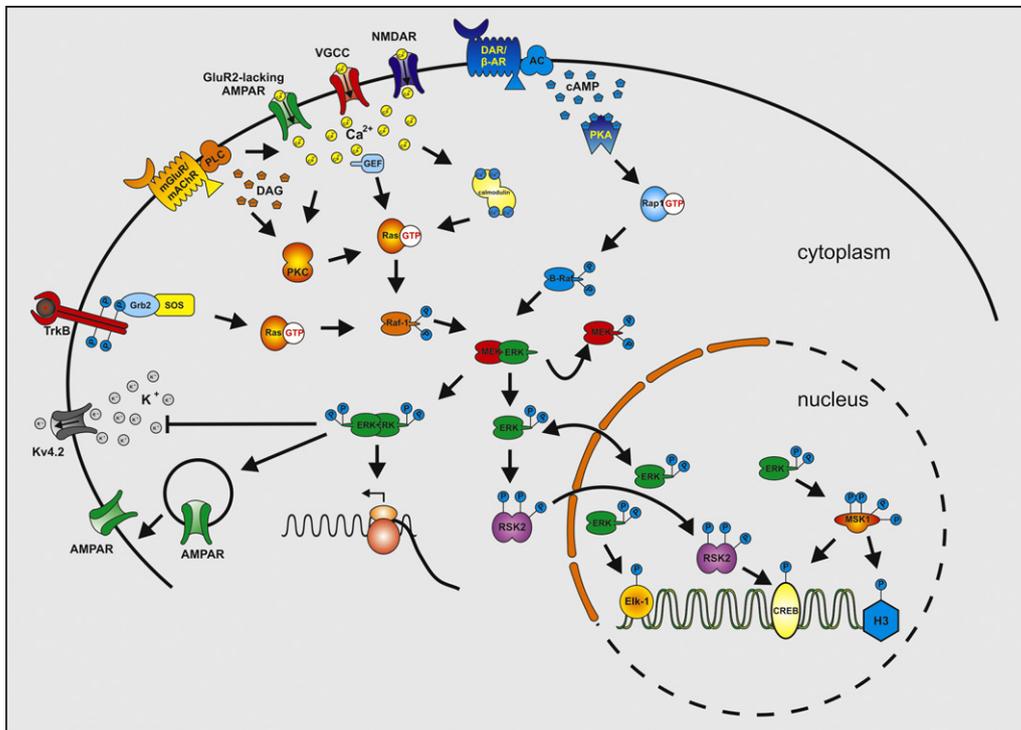


Fig. 1. Schematic overview of the major ERK1/2 signaling pathways in neurons. The regulation of the ERK1/2-MAPK cascade and its major downstream targets in neurons is schematically displayed. Many intracellular signaling pathways are integrated at the level of the ERK1/2-MAPK cascade in neurons of the central nervous system. These signals are induced at the level of many different receptors and ion channels, including NMDA receptors, GluR2-lacking AMPA receptors, voltage gated calcium channels, ACh receptors, metabotropic glutamate receptors, TrkB and several others. Within the cytoplasm, calcium ions, PKA, PKC and SOS directly or indirectly activate small GTPases such as Rap-1 or Ras, which in turn induce a release of the 14-3-3 protein from different isoforms of Raf, leading to their subsequent phosphorylation. The Raf isoforms then phosphorylate MEK1/2, which subsequently phosphorylates ERK1/2. Phosphorylated ERK1 and ERK2 are released from MEK1/2 and either dimerize with a second ERK molecule or remain monomeric. ERK1 and ERK2 can probably enter the nucleus in both forms. The mechanisms of nuclear import are not yet fully resolved. The double-headed arrow indicates that, in hippocampal neurons, facilitated diffusion of ERK-monomers is the prevailing mode of nuclear translocation. Within the cytoplasm, phosphorylated ERK1/2 regulates many processes, including attenuation of the A-current, activation of local gene translation, AMPA receptor trafficking or phosphorylation of RSK2. In the nucleus, ERK1/2 is primarily involved in the regulation of gene transcription. It phosphorylates Elk-1 and other TCFs, but also MSK1, which in turn modulates chromatin remodeling via the phosphorylation of histone H3. A further, indirect nuclear ERK1/2-target is CREB, which is phosphorylated by the ERK1/2-substrates RSK2 and MSK1.

scription with widespread importance for LTP, memory formation, epilepsy, drug addiction, depression and neurodegenerative diseases. In general, chromatin remodeling occurs via the acetylation, methylation, and phosphorylation of histones at their N-terminal tails [94–96]. These modifications allow specialized enzymatic protein complexes to bind to and uncondense chromatin making gene promoters accessible to the transcription machinery. One well-characterized example for the importance of histone acetylation in the nervous system is the co-activation of CREB-mediated gene transcription by the histone-acetyl transferase, CREB binding protein (CBP/p300) [43]. Despite the vast number of regulatory sites on histone tails it was recently demonstrated that ERK1/2-dependent acetylation of lysine 14 and phosphorylation of serine 10 on histone H3 were necessary and sufficient for the activation of the nucleosomal response at the promoters of the IEGs *c-jun* and *c-fos*. Moreover, in striatal neurons phosphorylation of serine 10 alone is sufficient for the activation of these promoters [97,98]. Importantly, independent studies confirm chromatin remodeling at the promoters of IEGs such as *c-jun* and *c-fos* as a major regulatory mechanism of their transcription [99]. Also here, the nucleosomal response involves the ERK1/2-dependent transcription factors such as Elk-1 or *c-Jun* [99]. Although in general the principal kinases which induce phosphorylation of serines 10 and 28 on histone H3 are p38 and ERK1/2 at the level of the MAPK family and MSK1/2 and RSK1–3 downstream of MAPKs [100,101] in the brain ERK1/2 and MSK1 are the key mediators of H3 phosphorylation [102,103,26].

This new form of ERK1/2-dependent regulation of IEG expression in response to neuronal stimulation is important in several physiological processes in the brain. In particular the nucleoso-

mal response is relevant in the hippocampus to the physiology of stress-related memory formation [104,105] and to contextual fear memory [106], while in the striatum, it is linked to the long-term effects of drug addiction and physiological reward-controlled learning [97,103,107].

Thus, playing a pleiotropic role at the level of gene transcription regulation, nuclear signaling of ERK1/2 appears to be more complex than it was originally thought. On the one hand nuclear ERK1/2 is important for direct or indirect phosphorylation of transcription factors such as Elk-1, CREB, *c-Jun*, *c-Fos* and others; on the other hand nuclear signaling of ERK1/2 regulates chromatin remodeling via MSK1 and histone H3 phosphorylation. This extended diversity of nuclear ERK1/2-signaling emphasizes the importance for efficient nuclear translocation of pERK1/2 in neurons.

5. Nuclear translocation of ERK1/2

Understanding the trafficking and nuclear translocation of ERK1/2 in neurons from synapses to the nucleus is particularly important in the light of its physiological relevance for plasticity and learning. However, despite a large body of evidence for ERK1/2 functions in neuronal signal integration and propagation a unified model describing the mechanisms that mediate trafficking and nuclear translocation of ERK1/2 in neurons does not exist.

In order to phosphorylate transcription factors, ERK1/2 has to propagate a signal to the cell nucleus from its site of activation, which in the case of synaptic activity is the submembranous space in the immediate vicinity of synaptic NMDARs [108]. Long-distance

communication may be accomplished either by active transport or by a diffusion process. It was demonstrated that active Ras can laterally diffuse within the plasma membrane of COS-1 and PC12 cells [109] resulting in a global Ras signal upon strong and prolonged extracellular stimulation. In pyramidal neurons, the lateral spread of Ras activated at single spines followed the pattern of passive diffusion and was limited to an area of approximately 10 μm along the length of the dendrite which lead to facilitated potentiation of neighboring spines [110]. This local confinement of the Ras-MAPK signaling module and the absence of active translocation towards the soma indicate that ERK1/2, activated at single synapses, preferentially acts near the site of synaptic activity where it may regulate local processes such as mRNA translation, spine enlargement, and AMPAR trafficking. Signal translocation from synapses to the nucleus could also involve endocytosis and signaling endosomes, similar to what has been described for nerve growth factor (NGF)-induced retrograde ERK1/2 signaling in axons [111–114] or TrkB-mediated neuronal protection and dendritic outgrowth in hippocampal neurons [115]. Indeed, endocytosis has recently been suggested as a key event in glutamate-induced nuclear translocation of ERK2 in cultured striatal neurons [116]. This signaling endosome hypothesis suggests an active mechanism to mediate synapse-specific retrograde nuclear signaling of small synaptic pools of pERK1/2. However, it remains to be shown how ERK2 is released from endosomes into the nucleus, and how selective activation of synapse-specific gene transcription is achieved. Whether pERK2-containing signaling endosomes occupy a dynein-mediated pathway along microtubules as suggested for the TrkA-containing endosomes remains elusive. Importins – the adapter molecules required for NLS-dependent nuclear import – also interact with dynein suggesting that microtubules may form the tracks for the retrograde dendritic transport of several synaptic molecules (e.g. Abi-1, Jacob and CREB2) that are imported to the nucleus in an NLS-dependent manner. In contrast, nuclear translocation of other nucleocytoplasmic messengers (e.g. AIDA-1) does not require microtubules [20]. An alternative pathway from synapses to the nucleus is based on lateral diffusion along the ER membrane. However, direct evidence for retrograde transport of activated nuclear messengers from the synapse into the nucleus via any of these routes is still missing to our knowledge. Apart from transport or diffusion along defined axes, signaling molecules can also propagate by undirected diffusion. Efficacy and rate of diffusion may be determined by phosphorylation of import signals and adaptors, cytoplasmic anchoring, proteolytic cleavage and calcium levels in an activity-dependent manner [20].

Diverging theories about how ERK1/2 translocates across the nuclear envelope largely reflect the different strategies employed by different cell types. Findings in a variety of non-neuronal cells and cell-lines suggested several modes of nuclear ERK1/2 translocation that range from active import mechanisms [117–119] to passive diffusion [119–121]. Notably, nuclear signaling in non-neuronal cells may be differently regulated since ERK1/2 plays a key role in development, differentiation and proliferation employing numerous sets of genes that may require specific ERK1/2-signaling patterns. Since neurons are terminally differentiated and no longer divide, the functions of ERK1/2 are shifted towards other tasks largely concerning the regulation of plasticity. According to the cell type and its specific requirements for ERK1/2-signaling nuclear localization of ERK1/2 may be actively regulated by import receptors [117–119,122] that may or may not require dimerization of ERK1/2 [120,123,124]. Despite an abundance of studies reporting (the requirement for) nuclear translocation of ERK1/2 during synaptic plasticity, learning and memory, little interest was paid to the underlying ‘import’ mechanism in neuronal cells [37,40,49,106,125,126].

Our group has proposed a mode of ERK1/2-signaling in hippocampal neurons where ERK1/2 activated at the synapse remains spatially confined and acting only locally in the narrow range of its diffusional reach (see below) [26]. In this scenario, gene induction only takes place if neuronal activity is sufficiently strong to trigger action potentials causing a rise in somatic calcium through activation of VGCCs and calcium-induced calcium-release from the endoplasmic reticulum. This calcium rise would, in turn, lead to activation of ERK1/2 throughout the neuron, including the somatic/perinuclear pools which may subsequently enter the nucleus to phosphorylate transcription factors and nuclear localized kinases [127] (Fig. 2). This is supported by the observation in hippocampal neurons that nuclear translocation of active ERK1/2 occurs via facilitated diffusion and that diffusion within the cytoplasm is not spatially directed [26]. Activation of perinuclear ERK1/2-pools might as well take place without the need for action potentials if synaptic input occurs sufficiently near to the soma. However, this scenario is unlikely for pyramidal neurons, given that most somatic synapses are inhibitory.

Unlike calcium/calmodulin, ERK1 and ERK2 exceed the size limit for passive diffusion through the nuclear pore and therefore rely on direct interaction with the nuclear pore complex in order to traverse the nuclear envelope [128]. Operating independently of import adapter proteins and energy, facilitated diffusion follows a concentration gradient whereas active import selectively accumulates cargo in the nucleus. Moreover, in its inactive state ERK1/2 is tightly bound to its upstream kinase MEK1/2 which is constantly shuttled out of the nucleus by an active export mechanism [129]. Thus, inactive ERK1/2 does not accumulate in the nucleus; however a somatic perinuclear calcium signal sufficient to phosphorylate ERK1/2 releases it from its bonds to MEK1/2 making ERK1/2 available for facilitated diffusion to the nucleus. The amplitude and kinetics of somatic calcium signaling activating the MAPK cascade in the perinuclear space are determinant in releasing a sufficiently large perinuclear pool of ERK1/2 from MEK1/2 to generate an efficient nuclear signal. Hence, nuclear translocation is efficient for somatic populations of phosphorylated ERK1/2 that are close to the nucleus whereas more distal portions may reside within the area of their activation to act on local cytoplasmic targets (Fig. 2).

6. Plastic changes in nuclear geometry

Facilitation of calcium signaling within specialized compartments of neurons is essential to neuronal plasticity. One of the best known examples is the dendritic spine which can generate highly localized, supralinear calcium transients that lead to activation of many intracellular signaling pathways [130–132]. Due to its geometry the spine can integrate electrical signals very efficiently. Moreover, strong depolarization of the spine can induce morphological changes in the spine head and neck improving its capability to generate a large and highly localized calcium signal [130–133]. Constriction of the spine neck restricts diffusion of synaptic signaling proteins (e.g. alphaCaMKII) to the spine head and may even electrically isolate the spine from the dendrite leading to non-linear amplification of voltage/electrical signals [133–135]. Activity-induced morphological changes in spine size and geometry demonstrate how neuronal activity can modulate calcium signaling in a defined subcellular compartment by altering its shape. A second, well described example for structural plasticity at a more macroscopic level is the activity-dependent rearrangement of dendritic geometry, which will change the network integration of the neuron [2,136]. Such morphological modifications represent a form of ultrastructural plasticity that provide a mechanism by which electrical and synaptic activity can modify signal integration and information processing.

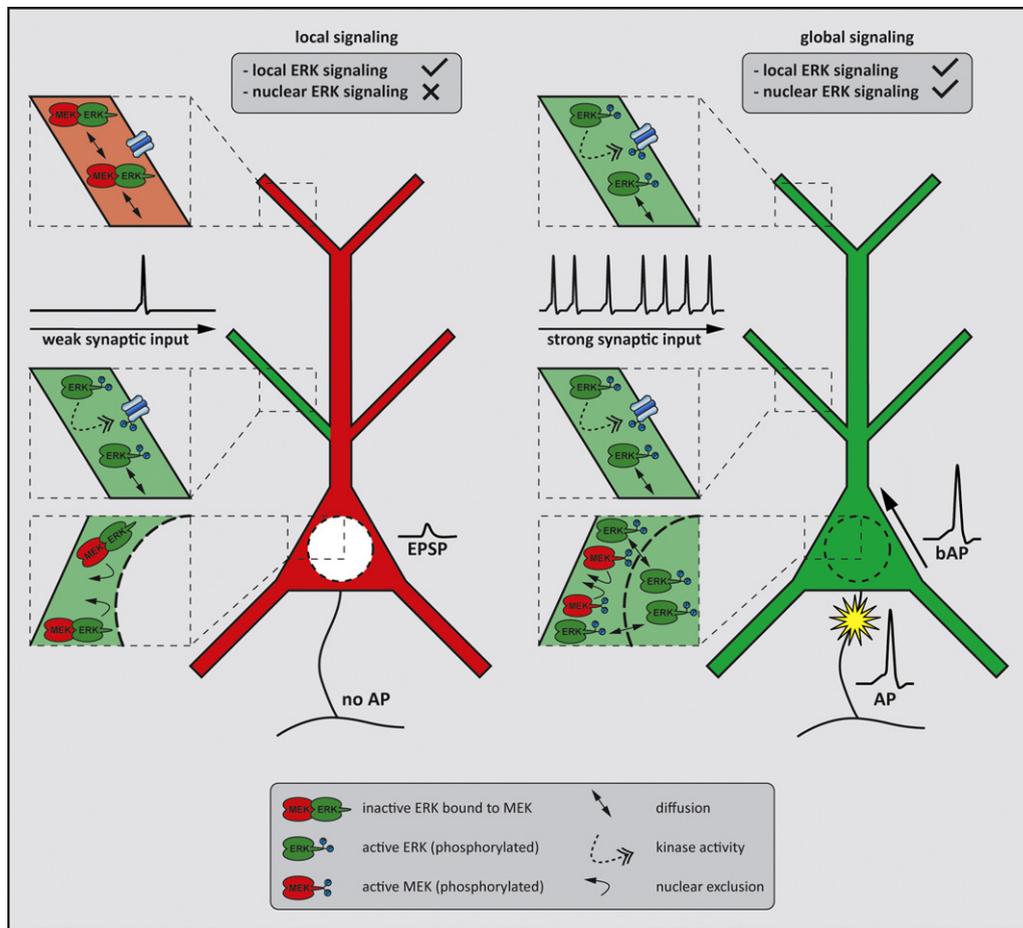


Fig. 2. Activity-dependent translocation of ERK1/2 in hippocampal neurons. The dendritic and somatic distribution of inactive and active (i.e. phosphorylated) ERK1/2 is dependent on the location and the intensity of electrical activity. Local activity at a small number of synapses in a distal dendrite will activate only the nearby population of ERK1/2, leading to spatially confined signaling events. This pool of pERK1/2 (represented by the green filling of the neuron) will dilute along the length of the dendrite and may not reach the nucleus in concentrations sufficient for the activation of gene transcription. Most ERK1/2-molecules in the soma and around the nucleus remain unphosphorylated and bound to MEK1/2 (represented by the red filling of the neuron). In contrast, strong synaptic activity that leads to the initiation of back propagating action potentials leads to phosphorylation of ERK1/2 throughout the entire cell resulting in a large population of somatic pERK1/2, which can translocate into the nucleus.

To date, research in the field of neuronal plasticity has mostly considered morphological or ultrastructural plasticity in terms of changes at the spine such as changes in active zone or PSD size or spine sprouting with little consideration given to structural modifications elsewhere in the cell. As extensively discussed throughout this review the nucleus represents a major subcellular compartment involved in the integration of a multitude of calcium signals. Hence, it would be conceivable for nuclear signaling events to be facilitated by some form of structural adaptation induced by neuronal activity. Our group recently addressed this question with an analysis of nuclear ultrastructure in hippocampal excitatory neurons. Interestingly, in these cells, the nuclear envelope proved to be highly plastic, forming deep invaginations and nuclear signaling microdomains upon strong synaptic activation [137,138]. This striking reorganization of nuclear geometry was dependent on calcium entry through synaptic NMDARs and was opposed by the activation of extrasynaptic NMDAR signaling pathways that promote cell death [139,140]. Moreover, inhibition of the MAPK cascade or blockade of synaptic activity or action potentials prevented the formation of nuclear infoldings.

Invaginations of the nuclear envelope have been observed in a wide range of mammalian cell types and in general are believed to enhance nuclear calcium signaling by allowing second messengers such as calcium to gain better access to deeper parts of the nucleus [141–144]. Despite the evidence for the presence of calcium release channels within nuclear infoldings it is not clear whether cal-

cium is released directly into the nucleoplasm [23,25,144]. Work in pituitary cells proposed a direct nuclear release mechanism [143]. The study by our lab demonstrates that infoldings in hippocampal neurons are contiguous with cytoplasm and therefore are not extensions of only the inner nuclear membrane into the nucleoplasm. However, if and how direct release from stores into the nucleus is affected remains to be shown.

What could be the implications of nuclear structural plasticity for the neuron? In general, two major aspects of calcium-regulated activity-dependent gene transcription may be affected: (1) activity-dependent facilitation of nucleoplasmic calcium signaling and (2) reorganization of chromatin and increased transcriptional activity (Fig. 3). Infolded nuclei feature a larger surface-to-volume ratio, an increased number of nuclear pore complexes and subnuclear compartments formed in nuclei where infoldings are deep. Our modeling studies predicted that calcium could penetrate into the nucleus with faster kinetics and reach higher intranuclear concentrations in infolded nuclei. Indeed, calcium imaging experiments revealed faster rise and decay times and increased peak calcium concentrations in infolded nuclei suggesting that deep infoldings generate calcium signaling microdomains in the nucleus that better reflect the kinetics of somatically generated calcium oscillations [137,138] (Fig. 3). Thus, in analogy to other neuronal compartments such as dendritic spines, synaptic activity may enhance nuclear calcium signaling on a structural level by physically reorganizing the shape of the nucleus and making its envelope more perme-

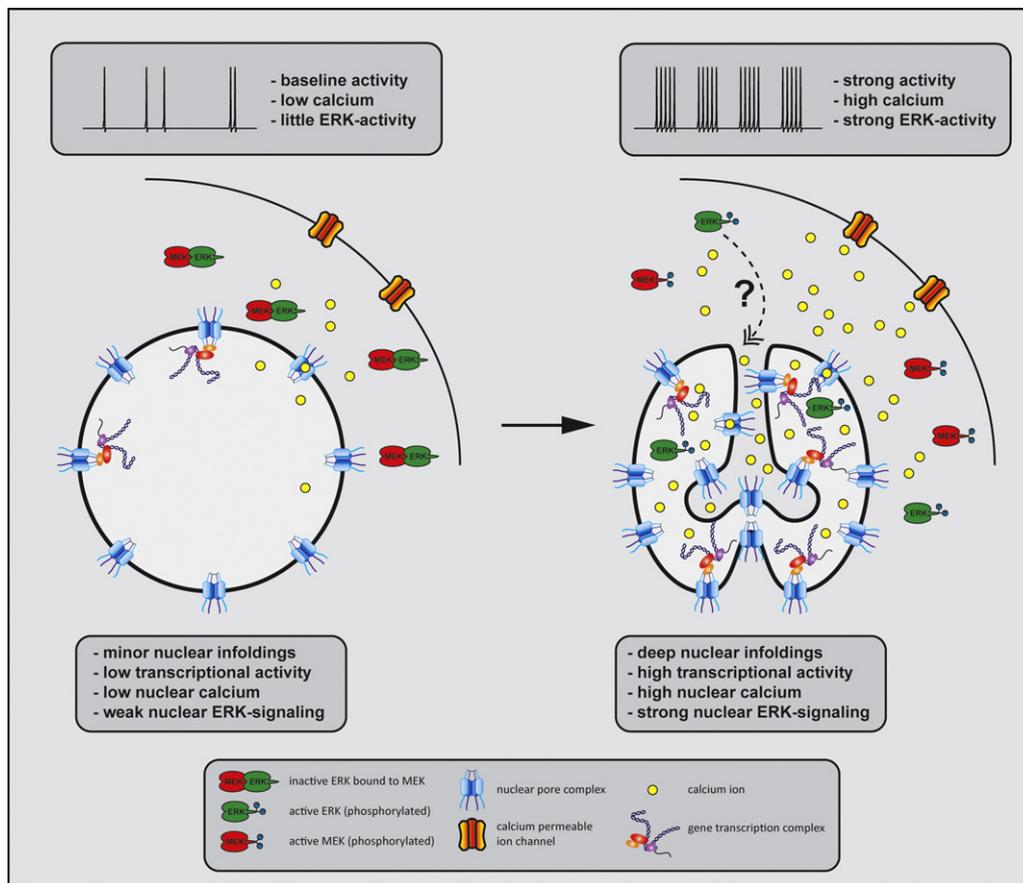


Fig. 3. Activity-dependent plasticity of nuclear geometry in hippocampal neurons – a hypothetical model. During baseline synaptic activity neuronal nuclei display no or only minor infoldings. Calcium signaling is at rest and most ERK1/2 is in its inactive (i.e. unphosphorylated) state and bound to MEK1/2. Upon initiation of strong electrical activity deep nuclear infoldings appear in an ERK1/2-dependent manner, which is depicted by the dashed arrow. The question mark indicates that the exact pathway that leads to this phenomenon is not known. The consequences of nuclear geometry changes are: larger surface area with more nuclear pore complexes, deeper penetration of calcium as represented by the yellow spheres within the nucleus, and increase in NPC-associated transcriptional events.

able for calcium. However, not only diffusion of calcium but nuclear translocation of signaling molecules in general may improve with increased numbers of nuclear pores which implies that also nuclear communication of all second messengers become more efficient.

Aside from enhanced calcium signaling, structural plasticity of the nucleus may go hand-in-hand with activity-dependent chromatin remodeling and altered gene transcription. Increased NMDAR-dependent synaptic activity was demonstrated to induce specific sets of genes and many of these genes require nuclear calcium and ERK1/2-activity for their transcription [145,146]. Such changes in the gene expression profile may require large-scale reorganization of chromatin in the nucleus. This scenario is supported by the view that sites of active gene transcription are localized to certain subnuclear territories [147] and that hotspots of transcription are found in conjunction with nuclear pore complexes [148–150]. Moreover, A-type lamins, which line the inner nuclear membrane, on the one hand, are major components of the nuclear lamina – a protein network controlling nuclear shape. On the other hand, they have been implicated in the regulation of gene transcription and chromatin organization [151]. Strikingly, lamin A/C can bind to pre-existing c-Fos and sequester it at the nuclear envelope [152]. Direct phosphorylation of lamin A/C by ERK1/2 releases c-Fos from the nuclear envelope and thereby enables c-Fos to form dimeric complexes with c-Jun and in this way to activate AP-1-dependent gene transcription in the nucleoplasm [151,152]. In view of these two examples for the involvement of nuclear membrane associated domains and protein complexes in the regulation of gene-transcription, it is conceivable that plastic modification

of the nuclear envelope provides a mechanism for altering gene-expression patterns. Such changes in the gene expression profile are induced by the induction of strong synaptic activity which is accompanied by transcriptional upregulation of a large gene-pool [146] which may reflect a metabolically more active state of the neuron. An increased number of NPCs in infolded nuclei could provide more anchoring sites for transcriptionally active promoter regions to bind to NPCs providing the cell with a means to adequately respond to the increased demand for gene-transcription (Fig. 3).

7. Closing remarks

Regulation of activity-dependent gene expression in neurons is a complex process occurring at many different levels in the cell ranging from local expression of a few proteins up to global expression of hundreds of genes. With ongoing research, new pathways are revealed by which the neuron may adequately control its protein composition. For example, the discovery that microRNAs are essential for post transcriptional regulation of genes involved in a large number of physiological processes adds another layer of complexity to neuronal gene expression regulation. Aside from the discovery of new pathways, previously unknown roles for established pathways are emerging. One example on which we laid our focus in this review is the mode of nuclear ERK1/2 signaling. The ERK1/2-MAPK cascade is at the hub of integration of a plethora of calcium dependent signaling events in the cytoplasm and nucleus. Thus, it is no surprise that this molecule is ascribed with an increas-

ing number of roles in neuronal physiology. One exciting new concept for activity-dependent gene expression regulation through ERK1/2 is based on large-scale structural changes inside the nucleus and of the nucleus itself. Chromatin remodeling and structural plasticity of the nuclear envelope provide the means to adapt gene expression programs on a large scale to changes in the activity state of the neuron. For example, a robust increase in synaptic activity and subsequent action potential firing will enhance the metabolic demand of the neuron requiring a timely upregulation of gene expression. The pathway by which ERK1/2-activity leads to histone H3-dependent chromatin remodeling is well-characterized. However, it remains to be seen how and if structural plasticity of the nuclear envelope is linked to chromatin remodeling. Moreover, the molecular targets of ERK1/2, which connect synaptic activity to changes in nuclear geometry, remain unknown. Recent discoveries in non-neuronal cells that directly link ERK1/2-signaling in the nucleus to the regulation of c-Fos interaction with the inner nuclear envelope provide the first hint that proteins comprising the nuclear envelope may be direct targets of ERK1/2.

Conflict of interest statement

There is no conflict of interest.

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